

Supplemental Materials

SARS-CoV-2 LDT

For the SARS-CoV-2 laboratory-developed test (LDT), 0.2 mL of the raw respiratory sample is combined with 2.0 mL of NucliSENS® lysis buffer (bioMérieux; Durham, NC) inside a certified class II biosafety cabinet. The lysed samples are subsequently extracted on the NucliSENS eMAG® (bioMérieux; Durham, NC) according to the manufacturer's instructions. The purified nucleic acid is then tested for SARS-CoV-2 using a TaqMan™ real-time PCR assay on the LightCycler 480 (Roche Applied Sciences, Penzberg, Germany). The SARS-CoV-2 LDT targets two regions of the SARS-CoV-2 genome, the nucleocapsid (NUC) and open reading frame (ORF) regions, along with a murine hepatitis virus (MHV) internal control (IC) target (Table S1). For each clinical sample, positive control, and negative control, 15 µL of NUC and ORF master mix containing the IC (Table S2 and S3) is added to separate wells of the LC480 plate. For each specimen, 5 µL of each sample extract is added to the appropriate wells. Positive and negative controls (5 µL each) are added to designated wells. The LC480 plate is sealed and centrifuged for approximately 30 seconds. The assay is performed using the cycling conditions outlined in Table S4 and analyzed on the FAM (NUC, ORF) or CY5 (MHV) channels. Samples positive for NUC alone or NUC and ORF are reported as presumptive positive, ORF alone as indeterminate, and negative if negative for both SARS-CoV-2 targets. The IC must be positive or the sample is reported as invalid. The limit of detection for the LDT is 156 copies/mL from NP swabs and 12,500 copies/mL from sputum.

Table S1. Primer and Probe Sequences

Target	Primer/Probe	Nucleotide Sequence (5'-3')
NUC	Forward primer	AGT CAA GCC TCT TCT CGT T
	Reverse primer	CTA CTG CTG CCT GGA GTT GA
	FAM labelled probe	FAM- CTT GAA CTG /ZEN/ TTG CGA CTA CGT GAT-3IABkFQ
ORF	Forward primer	AAT GAA TCT TAA GTA TGC CAT T
	Reverse primer	TAC AGA TAG AGA CAC CAG C
	FAM labelled probe	FAM - AAT AGA GCT /ZEN/ CGC ACC GT-3IABkFQ
MVH	Forward primer	TTC TCT GCC AGT GAC GTG
	Reverse primer	CAT TTG AAG CCG AGA CCG TA
	CY5 Labelled probe	Cy5-CAG CCC ACC CAT AGG TTG CAT-3BHQ_2

3IABkFQ and 3BHQ_2; quenchers from Integrated DNA Technologies (IDT, Coralville, IA)

Table S2. NUC Master Mix

NUC Master Mix		
Ingredient	Mix Concentration	Volume (μL)
Water	-	2.94
SuperScript 2X Mix	1x	10
SuperScript Enzyme	0.05x	1
IC	5x	0.1
NUC-F	0.3 uM	0.12
NUC-R	0.6 uM	0.24
NUC-P	0.3 uM	0.12
MVH-F	0.3 uM	0.12
MVH-R	0.6 uM	0.24
MVH-P	0.3 uM	0.12

Table S3. ORF Master Mix

ORF Master Mix		
Ingredient	Mix Concentration	Volume (μL)
Water	-	2.94
SuperScript 2X Mix	1x	10
SuperScript Enzyme	0.05x	1
IC	5x	0.1
ORF-F	0.3 uM	0.12
ORF-R	0.6 uM	0.24
ORF-P	0.3 uM	0.12

MVH-F	0.3 uM	0.12
MVH-R	0.6 uM	0.24
MVH-P	0.3 uM	0.12

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32 Table S4. SARS-CoV-2 LDT Thermocycling Conditions

Initial	PCR	Cool
Cycles: 1 Analysis: None 55°C x 20:00 @ 4.4°/s 95°C x 02:00 @ 4.4°/s	Cycles: 45 Analysis: Quantification 95°C x 0:15 @ 4.4°/s 55°C x 0:45 @ 2.2°/s - AS 72°C x 0:15 @ 4.4°/s	Cycles: 1 Analysis: None 40°C x 0:30 @ 2.2°/s

33 AS, signal aquisition

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